SMOKE INHALATION APPARATUS

Although several laboratories have published information on the response of the lung following the experimental exposure of animals to tobacco smoke, most of these results are controversial and subject to considerable criticism. The primary reason that experimental smoke inhalation studies have been so unsuccessful in the past is that there have been no smoking machines that could create a stable aerosol of either tobacco or marijuana smoke, comparable to that created by man when he smokes these products, that could be delivered to the lungs of experimental animals. It is our evaluation that all existing commercial machines now used fail in this crucial requirement. To solve this problem, we have built a total of four Lorillard smoking machines for our laboratory. This machine was initially developed primarily by the tobacco industry.

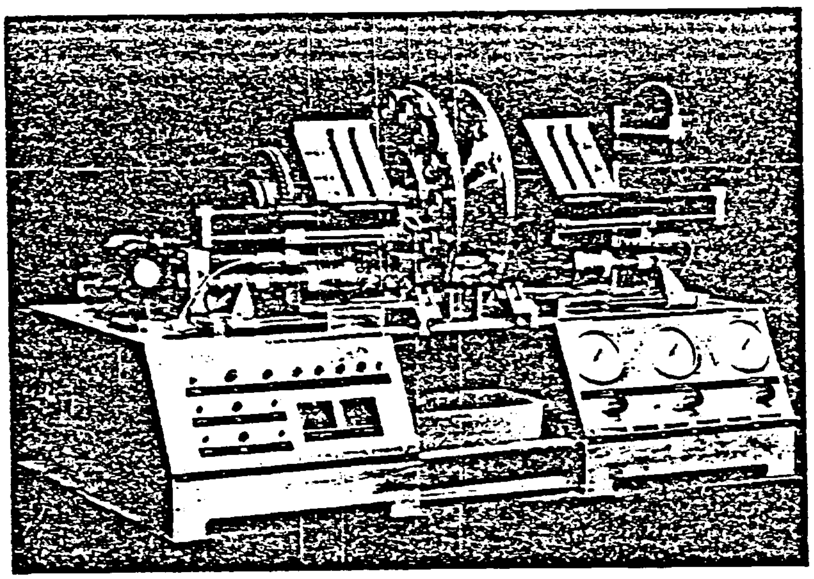

Figure X-1: The Lorillard smoking machine, capable of generating a stable cigarette smoke aerosol for delivery to the lungs of experimental animals.

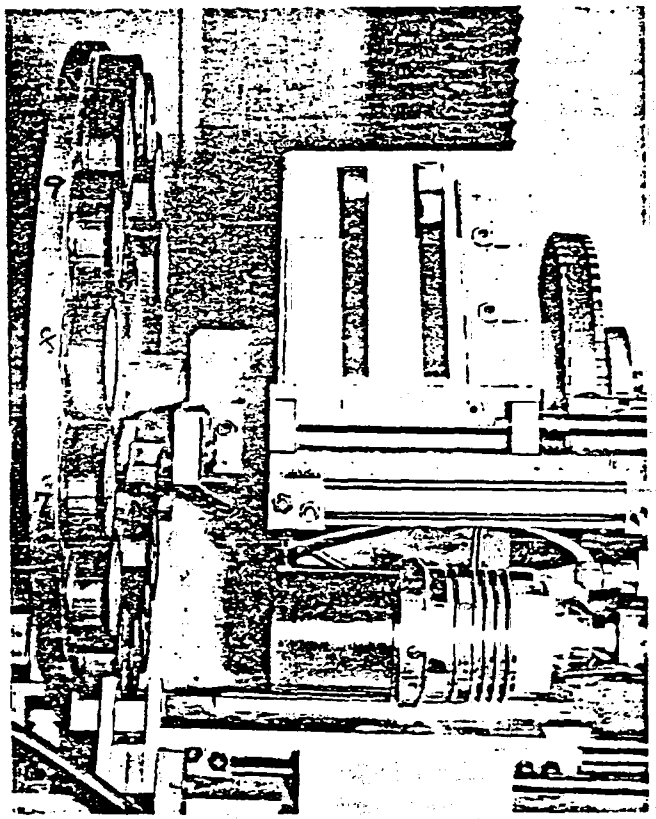

Figure X-2: A view of the loading cassette of the smoking machine. There are 30 portals for cigarettes, with 15 on each of the two rotating wheels. Cigarettes are automatically loaded and ejected after a predetermined number of puffs, usually 10. All cigarettes are conditioned with standardized humidity and temperature prior to use and burned, for the most part, to a consistant butt length.

One such smoking machine is shown in Figure X-1. These are very complex pieces of equipment and now cost over $30,000 each to construct. They have the capacity to expose approximately 150-450 experimental animals to various concentrations of tobacco or marijuana smoke per hour. Once operational, they appear, in our experience, to be able to run for literally months, with a minimum of maintenance or breakdown.

In the development of a smoking machine for the delivery of fresh smoke at ambient pressures to animal exposure systems, several design parameters are dictated if a wide range of exposure conditions are to be obtainable. Reverse smoking (the creation of a pressure around the lighted end to generate smoke exiting through the delivery system) is necessary to fulfill the requirement for fresh smoke at ambient pressure. A 30 port design resulted from the standard smoking conditions of a 35 ml puff with a two-second time duration once per minute. The 30 ports are logically broken into two groups because of the positioning, dwell, and reaction times associated with this system. A rotary positioning arrangement was preferred, since the smoking cycle is repetitious and the time cycle is fixed. Further, the cigarettes and delivery system should rotate in preference to the reverse smoking chamber(s), since this avoids rotary valves or cumbersome tubing arrangements.

Reverse smoking entails several difficulties which are not encountered where smoke is generated by pulling a vacuum on the cigarette. It is more diffucult to ensure the delivery of the correct volume of smoke. An accurate flow control system is mandatory and this will produce a nearly square puff profile, but it is difficult to decide upon the proper flow rate of the input air for puff generation. Not all cigarettes have the same pressure drop, and this will increase to a variable degree as a cigarette is lit and puffed, although all may have the same pressure drop initially. Pressurized air in the chamber is exhausted at the end of each puff, and the quantity is dependent upon the size of the chamber and the magnitude of the pressure drop. The combustion of the cigarette adds to the total volume generated during each puff and may vary from cigarette to cigarette. These experimental difficulties may be compensated for by careful design. Although reasonable estimates can be made for all factors, careful calibration with test cigarettes is needed, and this is done in our unit on a regular basis.

Further design criteria are a natural result of the foregoing requirements. There is, in our machine for example, the need for a smooth, stepwise rotary motion to position the cigarettes. The geneva movement provided an ideal solution, since it is very smooth and additionally offers dependability, low wear, accuracy of positioning and timing, and can be made rugged and maintenance free.

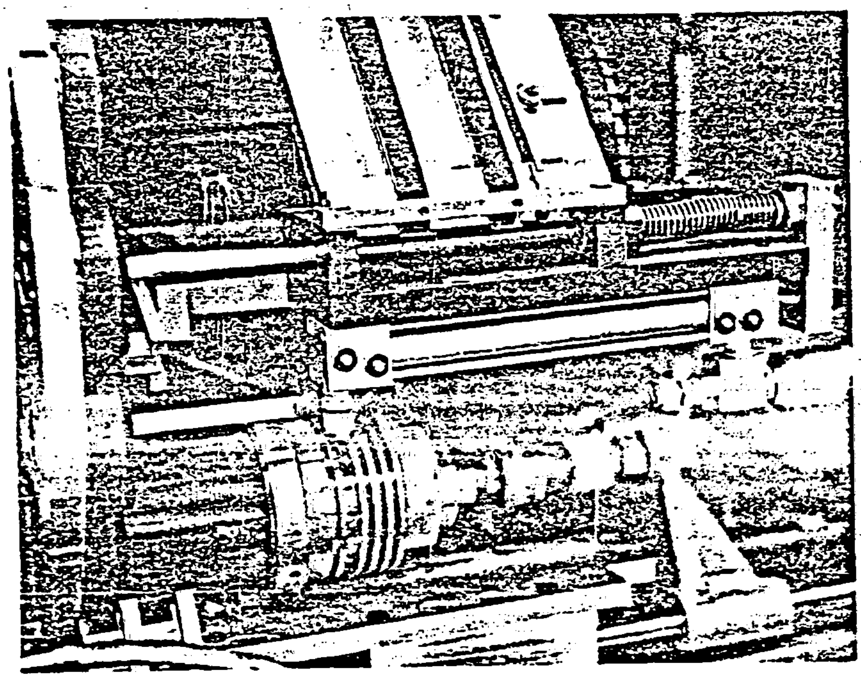

Figure X-3: Shown in this photograph is a view of the cigarette being loaded into a portal. This must be done under slight pressure. Tobacco cigarettes, that are tightly packed, immediately reform their original shape, as shown in the lower of the two cigarettes seen above.

In our machine the cigarettes are loaded into a cassette, as shown in Figure X-2 and automatically supplied to the cigarette holders as shown in Figure X-2 and Figure X-3. Once loaded, the puff chamber closes on the rubber cams and a standard amount of tobacco smoke is generated, created as a stable aerosol, which remains stable until delivery to the lungs of the experimental animals. Shown in Figure X-4 is the puff-chamber approaching the loaded and burning cigarette, and shown in Figure X-5 is the puff-chamber tightly closed around the rubber cam and the cigarette, delivering its puff that creates the stable exposure aerosol. The smoke generated is then passed through a dispersion system and a series of tubes. These are special conductors that do not allow the aerosol to precipitate on their walls in any significant manner and permit delivery of the generated smoke to the lungs of the animals in less than 0.2 seconds.

Air was chosen as the motive force for linear motion, since a rather long stroke was necessary to position a chamber over the cigarette, and for positive action and speed of response the stroke had to be powered in two directions. Since the machine had to be divided into two smoke-generating sections the shuttling action needed to couple them to the exposure system was easily accomplished.

A single timing source for all functions was desired, since this precludes any loss of synchronization. In conjunction with this requirement was the need for an efficient system of interlocks to prevent damage, and to ensure that the system, if not in proper operating condition, will not operate at all.

All timing originates from a synchronous motor. This motor serves a dual purpose in that it is coupled through a gearing mechanism to a four-position geneva movement and to the shaft of a sequence programmer. The motion of the geneva movement is transmitted through a notched timing belt to index the cigarette holders.

Ten pounds of pilot air pressure is supplied by a regulator to the valves of the sequence program. The cams on this programmer actuate the four control valves which power actuators on the four-way valves, which in turn control the motion of air cylinders on the puff chamber and the shuttle valve. An additional regulator supplies the 45 pound control air which will power both the puff chamber air cylinders and the shuttle valve. Should this air system fall below 40 psi the pressure switch would interrupt the operation of the machine.

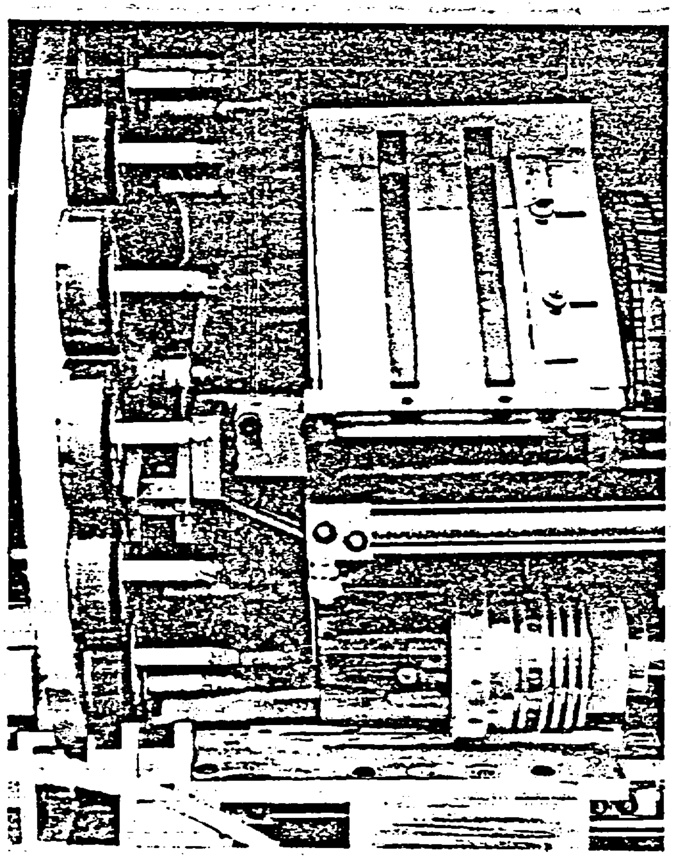

Figure X-4: Cigarettes are in place and burning in each portal. The only smoke generated under these conditions is the side-stream component. The puff-generating chamber is withdrawn in this position, allowing an automated programmed rotation of the cigarettes between smoking.

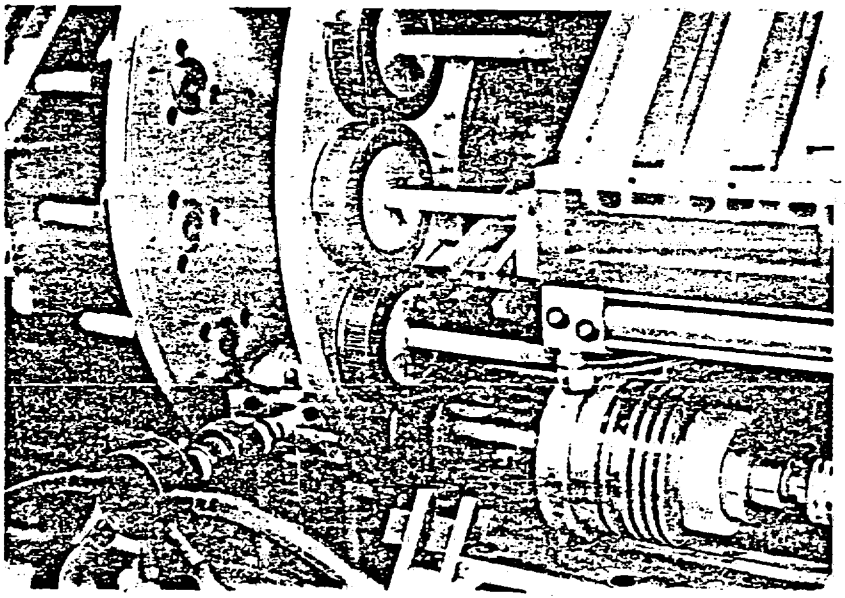

Figure X-5: The puff-chamber is closed on the rubber cam and generating 35 milliliters of fresh cigarette smoke. The puff volume and generation time can be programmed to any desired criteria.

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The cigarette smoke, once generated, is rapidly distributed to the conducting tubes and exposure chambers, as shown in Figure X-6 and Figure X-7. Although the delivery system could be expanded, we are comfortable with our current capacity to expose 50 rats at one time in each smoking machine. For our pigeon exposures, we have used a shuttle-valve and a full-loading complement of cigarettes to expose three, rather than one, carousels during each sequence.

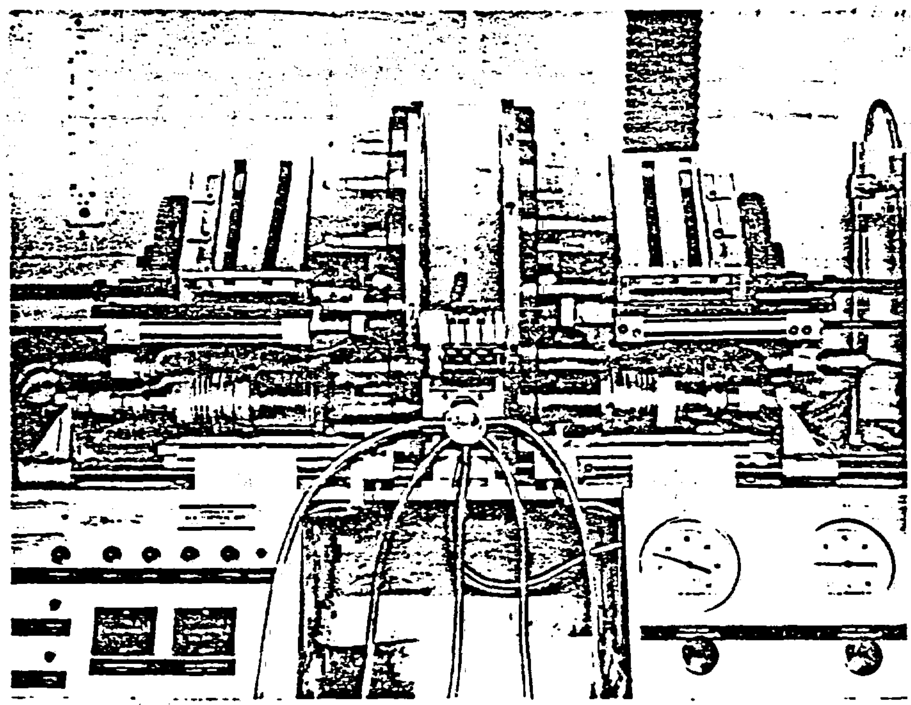

Figure X-6: Frontal view of the smoking machine in operation, with the five delivery tubes shown anteriorly. The puff-chamber on the right is generating fresh smoke and the one on the left is in an intermediate position.

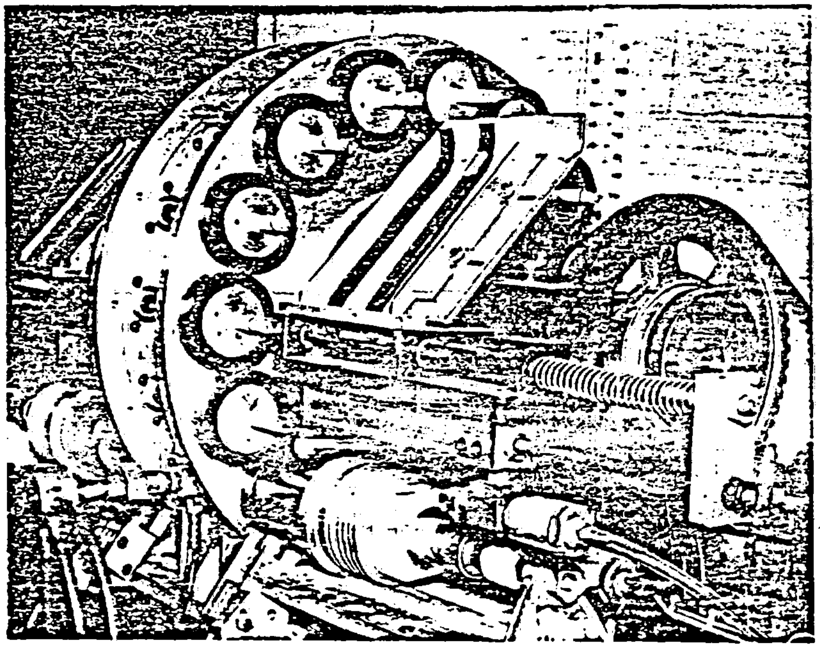

Figure X-7: An oblique view of the smoking machine with the dispersion conducting system.

Shown in Figure X-8 is the carousel used to hold the exposed rats, with the delivery or conducting tubes at the top and the exhaust system at the bottom. The flow of the aerosol is rapid and does not allow agglomeration or condensation of the fresh smoke aerosol nuclei in any significant degree. As can be seen, fifty rats can be exposed simultaneously in this system. The animals are held in small containers, shown in greater detail in Figure X-9 and Figure X-10. These containers involve an interlocking system for efficient and rapid loading and placement in the chamber. Once in place, the animals are relatively comfortable and inhale the smoke product as it passes through the exposure system. A very significant portion of our efforts in the funded period to date has been spent perfecting this smoke-exposure system and reducing the level of stress in the exposed animals to an absolute minimum.

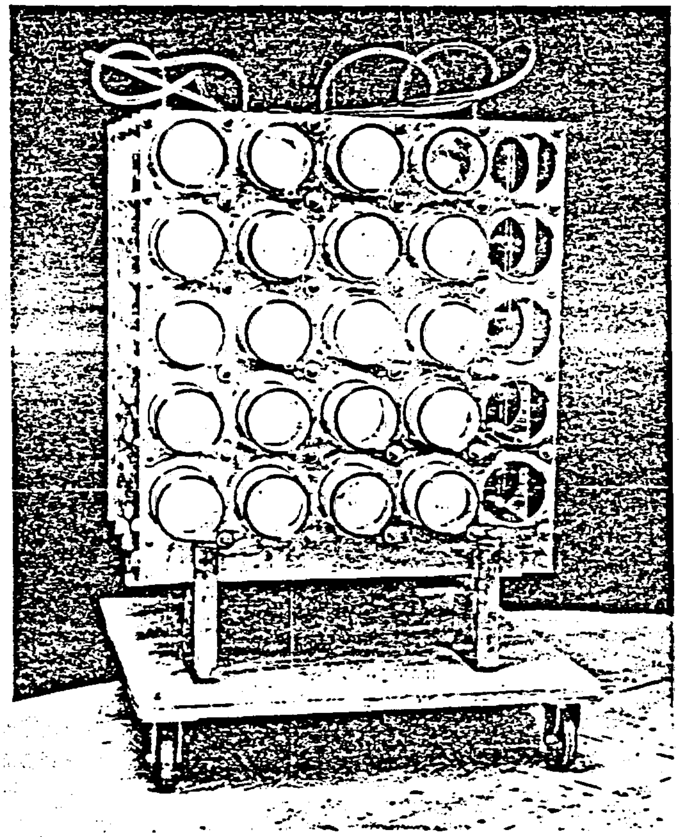

Figure X-8: The smoke-exposure carousel. Fifty rats can be exposed at one time. Each carousel is mounted on wheels for mobility and easy transport of animals from their housing to the exposure room and the smoking machines.

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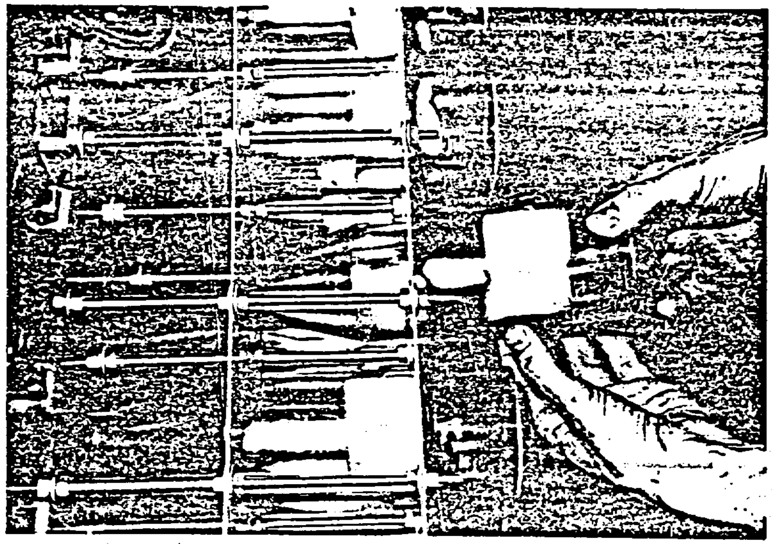

Figure X-9: The animal-holding chambers are transparent and can be easily removed from and placed in the exposure carousel. Animals are comfortably held in place by the sponge-rubber plug in the rear of the chamber.

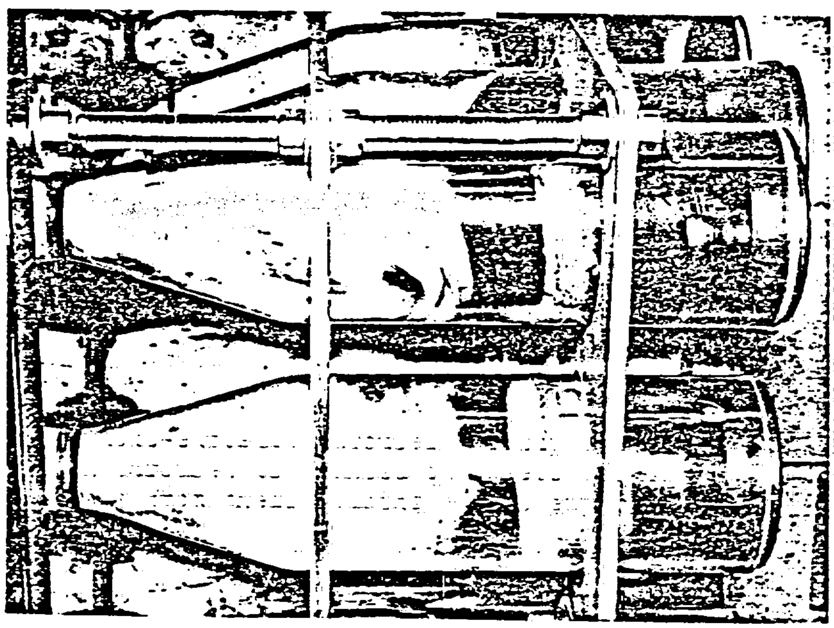

Figure X-10: A rat in place during exposure to smoke. This delivery system has the versatility to expose any sized animal, from the smallest rodent to an animal the size of a horse or larger, to the same stable aerosol of fresh smoke.

Our pigeons are exposed with a different system. We believe it is essential for the success of the experimental inhalation, that the pigeons be immobilized during smoke exposures to prevent them from putting their head under a wing (thereby screening out much of the particulate phase). We also believe that only the beak and nares should be exposed to the fresh smoke to avoid soiling the pigeon plumage with tar. Otherwise, the birds will injest substantial amounts of smoke products when they preen their feathers. These factors could be minimized with the hood and glottal cannula system, but as stated above we believe intubation to be impratical for long term studies of large number of pigeons, not only because of high man-power requirements, but, more importantly, because we believe repeated intubation would result in excessive premature losses of birds from physical injuries over the period of the proposed study. We are, therefore, developing a new method of exposing pigeons to tobacco smoke that is very gently and which will present smoke solely to the birds respiratory systems. Following closely the recommendations of Professor B. F. Skinner, we prepare the pigeons for their daily smoke exposures by immobilizing them in a closely-fitted cuff that could potentially be left around the birds for the duration of the total daily smoking period (6-8 hours). Professor Skinner, who has had extensive experience with these methods, has assured us that continuous confinement by this technique for the periods noted produces no discernable physical or physiological harm to the birds or any significant psychological stress. This system of immobilization could reduce in a major way the handling of the birds, thus reducing the cost of exposure and lessening the potential for accidental injury.

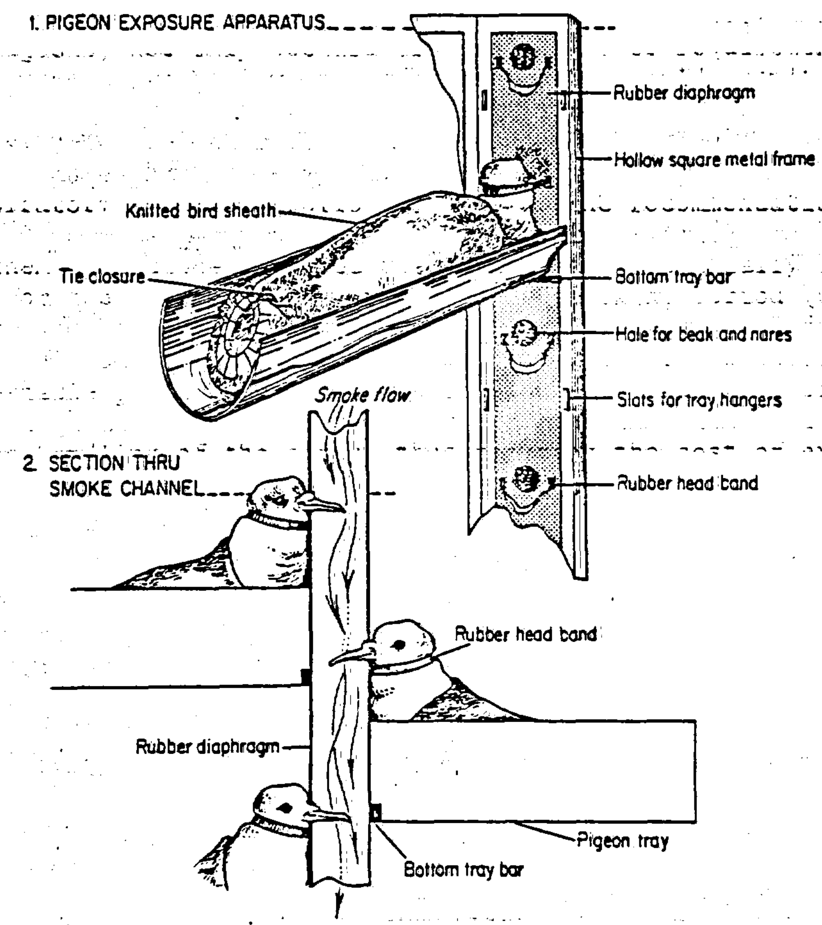

Figure X-11: Diagramatic illustration of the proposed method of immobilizing pigeons and exposing only their respiratory system, by noninvasive techniques, to cigarette smoking products.

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FRONT VIEW OF 60-PIGEON EXPOSURE RACK

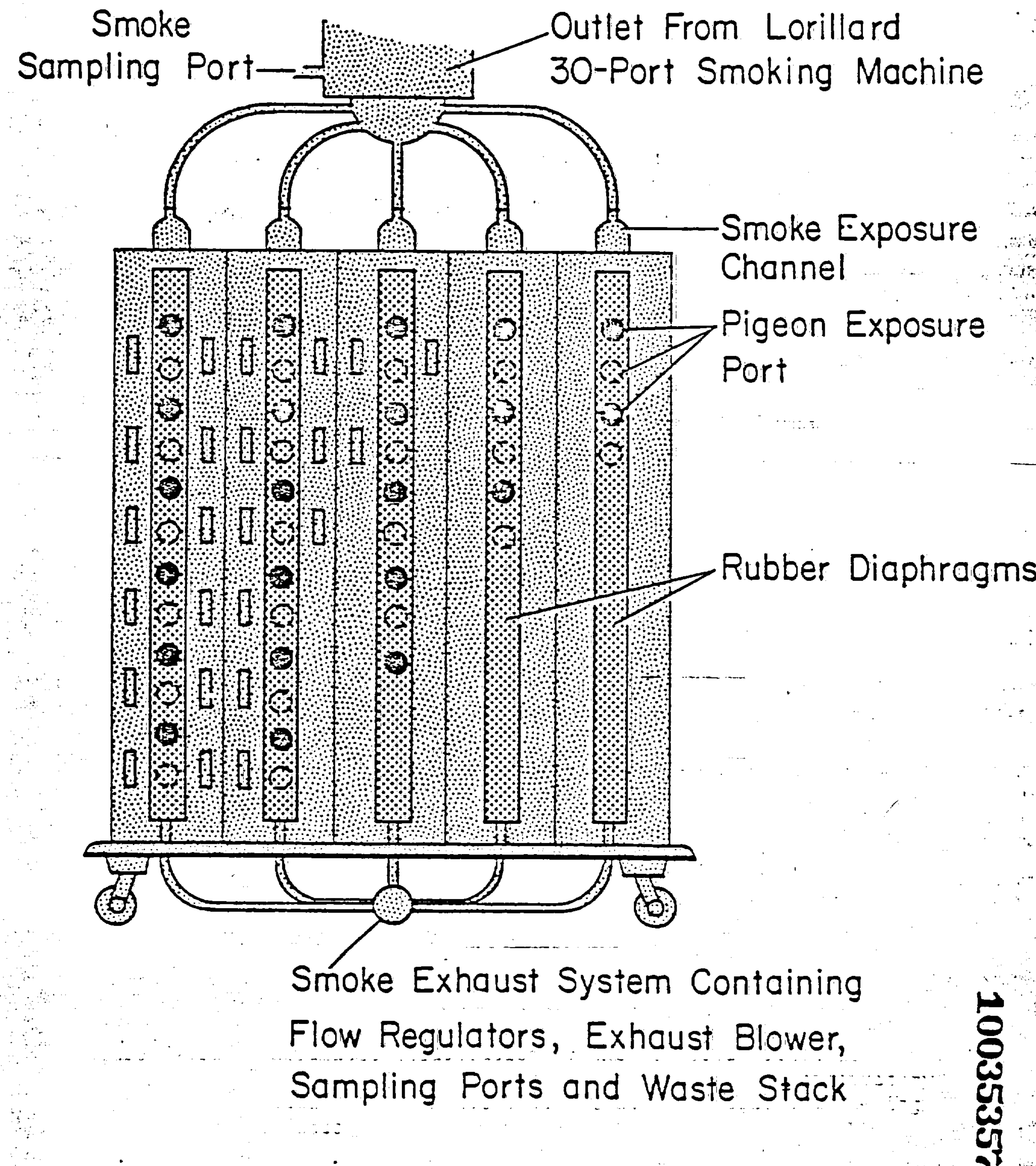

Figure X-12: Schematic diagram of the pigeon exposure rack.

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To expose only the beak and nares of the birds to tobacco smoke, we constructed an exposure apparatus containing a rubber diaphram with holes of appropriate size and locations through which the bird's beak, up to and including the nares, can be inserted to form a gas-tight seal. This is illustrated in Figure X-11 and Figure X-12. Tobacco smoke suitably diluted, or spiked with carbon monoxide, passes through a channel on the side of the flexible diaphram in which the beak and nares are located, while the remainder of the bird remains in clean laboratory air. A helmet attached to the diaphram is gently slipped over the bird's head to prevent it from withdrawing its beak from the smoking portal. Because the diaphram is soft and flexible, movements of the bird's head while exposed to smoke will neither produce injury nor break the firm seal between the smoke channels, on one side, and the laboratory atmosphere on the other.

To equate exposure levels (smoke delivered to the whole animals) for both our pigeon and rat experiments with dosimetry (actual amount of smoke inhaled), we have perfected a method of lacing all units with a nonradioactive chlorinated hydrocarbon, decachlorobiphenyl (DCBP). The lacing machine shown in Figure X-13 is used to evenly deposit tracer into a single cigarette. With this machine it is possible for one operator to lace 85 cigarettes per day with an accuracy, characterized by a relative standard deviation, of 1%.

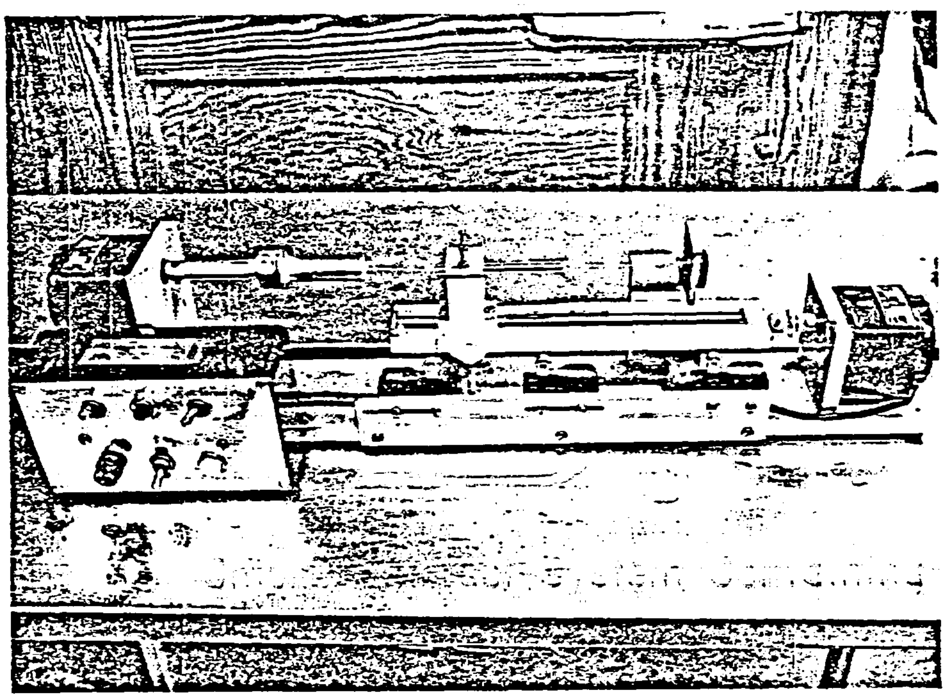

Figure X-13: Lacing machine.

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Figure X-14 shows and enlargement of the lacing machine cigarette holder. The cigarettes are manually placed in this holder. When the machine is switched on, the holder rotates the cigarettes slowly allowing for even deposition of tracer. Figure X-15 shows an enlargement of the syringe mechanisms of the lacing machine. Due to modifications of the machine the syringe is filled manually with DCBP tracer using chloroform as a solvent. The syringe assembly is then switched on to drive the 100 µl syringe into the cigarette. When the syringe needle reaches the end of the cigarette it is stopped automatically. At this point a butt plate is placed behind the plunger of the syringe and the syringe withdrawn, automatically depositing equal amounts of tracer throughout the full lingth of the cigarette.

By using this system, we can deliver "laced" smoke to the animals. Total "tar" in the delivery stream is quantified by the gravimetric methods and cross-calibrated with tracer content. The total amount of tracer in the lungs of each animal is quantified by chromatographic analysis. Then, by back calculating to the tar-lacer ratio in the smoke delivered, the exact amount of tar delivered to the lungs of animals can be accurately determined. By using these techniques, and by also simultaneously meansuring CO in the gas phase of the delivered smoke and HbCO in each animal, we have an accurate assessment of both gas phase and total-particulate delivery of smoke to each individual animal. From these measurements precise correlations between biologic effects and true dosimetry can be determined.

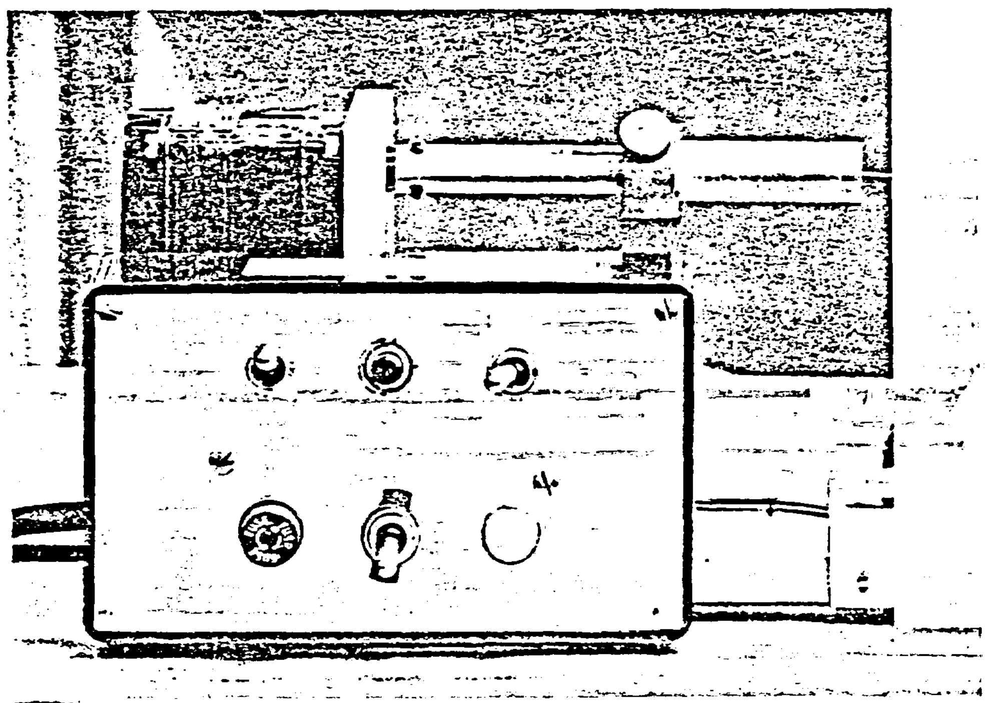

Figure X-14: Lacing machine cigarette holder.

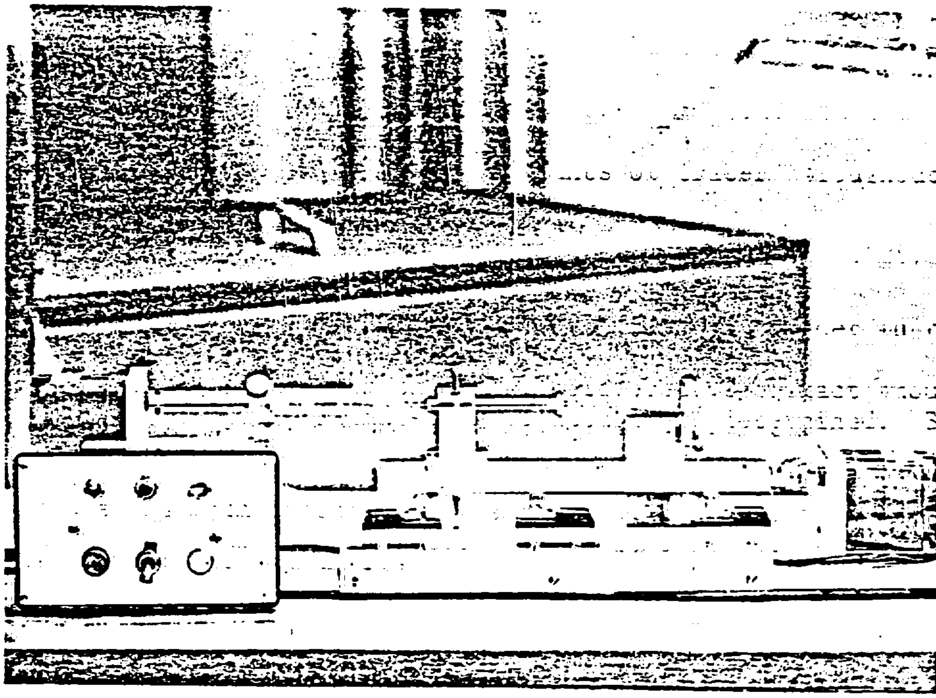

Figure X-15: Lacing machine syringe mechanism.